Measurement of human genomic DNA concentration using real-time Taqman PCR, or quantitative genomic PCR (QG-PCR)

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This assay involves measurement of human-specific genomic DNA concentration using Taqman technology. It involves amplifying a 119 bp fragment of non-amino acid coding human DNA (intron 11 of *BRCA1*) with a 27 bp probe labeled with 6-FAM at the 5' end and a Black Hole quencher on the 3' end. The assay is run on an ABI 7700 in real time and includes serial dilutions of a sample of 'known' DNA concentration from which a standard curve is derived, plotting the threshold cycle against the logarithm of input DNA. Test samples are plotted on the standard curve and the DNA amount is calculated. The assay is highly linear (based on serial dilutions of several samples) over 3-4 orders of magnitude of input DNA (typical standard curve inputs are 1 ng to 100 ng of genomic DNA), with R^2 values typically > 0.99. There is no detectable signal when using genomic DNA from *S. cerevesiae*, mouse, dog, chicken, cat, bovine, or oral flora as input.

Primer & Probe Sequences

Numbering is derived from Genbank record L78833.1

L78833:37423F	aaacatgttcctcctaaggtgcttt
L78833:37541R	atgaaaccagaagtaagtccaccagt
L78833:37462P	5'-FAM-ccttcacacagctaggacgtcatcttt-BH-3'

F & R primers synthesized by IDT with standard desalting purification.
Probe synthesized by Keystone Labs (now owned by Biosource International).

The final concentration of primers and probes were arrived at after optimization of the reaction to get the best looking log-linear amplification plots with the smallest amount of probe.

Genomic DNA standards

A cell line from CEPH individual 1347-02 was expanded and a large quantity of DNA extracted and measured several times using spectrophotometry and Picogreen to arrive at a "known" concentration.

Fairly large quantities of serial dilutions were then made to create a standard curve for each run. Standards typically used to construct the curve are:

Sample	Conc. (ng/ml)	ng per 4 µl
4200-10B	28,571	114.3
4200-10D	5,644	22.6
4200-10E	2,508	10
4200-10F	1,115	4.5
4200-10H	220	0.88

Software Settings

We run in 96-well trays on an ABI 7700 using SDS 1.7, with the following settings:
New Plate – Single Reporter; Standard Plate; Real Time
Sample Type Setup - FAM layer with all samples types set to either NTC or UNKNOWN; No Quencher

Thermal cycler conditions -

50^0C 2:00
95^0C 10:00
then 40 cycles
92^0C 0:15
60^0C 1:00

25 µl Sample Volume

Advanced Options – Set exposure time for plates – 10 (we use ABI optical plates and Optical Adhesive Covers; Spectral Compensation for Real Time OFF; ROX Reference

Analysis Options – Extension Phase Data Extraction – Automatically compute PCR stage

Master Mix set-up

n=1	Component	Final [ ]
12.5µl	2X Taqman Universal Master Mix (ABI)	1X
1.0 µl	Forward primer (10 µM)	400 nM
1.0 µl	Reverse primer (10µM)	400 nM
0.125 µl	Probe (10 µM)	50 nM
4.0 µl	gDNA	
6.375 µl	H_2O	
25 µl		

The 5 DNA standards are run in duplicate and test samples run singly.

We typically dilute a test sample to roughly 10ng/µl (if we can estimate this) and use 4 µl as template in the reaction – this readout – 40ng, falls nicely on the standard curve and this is a fairly comfortable pipeting volume, but other schemes would obviously work too.

We have taken to running an aliquot of the same sample on each run but have not yet taken to "calibrating" to this sample from run-to-run, but may do this.

Post cycling analysis includes setting the cycle threshold (C_t) manually midway up the log-linear portion of the amplification plots. We then export the results and use fairly simple Excel formulae to calculate the standard curve and "forecast" the input amounts of our test samples from this equation. The built-in SDS software can also be used to calculate input amounts.